Recovery of Normal Growth with Pulsatile Human GH Secretion in Spontaneous Dwarf Rats by Targeted Expression of the Human GH Transgene to the Pituitary Gland

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Abstract. A strain of spontaneous dwarf rat (SDR) with isolated complete GH deficiency of autosomal recessive inheritance (gene symbol dr) produces characteristics similar to those of a human disorder, isolated GH deficiency Type I. To characterize a 1.7 kbp 5' flanking promoter structure of the rat GH (rGH), we established a strain of hGH-transgenic SDR (PGGB dr) which bore a chimeric gene (PGGB) of the 1.7 kbp 5' flanking segment of the rGH gene fused to the human GH (hGH) gene (2.1 kbp) in SDR, and examined the hGH gene expression to recover normal growth. Both Northern blotting and RT-PCR analyses showed that the transgene transcript was expressed exclusively in the pituitary. In spite of the lactogenic action of expressed hGH, both the litter size and growth rate of PGGB dr were identical to those of age- and sex-matched non-transgenic littersmates. Sequential blood sampling from individual PGGB dr under light ether anesthesia at days 20, 40 and 50 after birth showed age-related increases in plasma hGH, as did plasma rGH levels in each of normal Sprague-Dawley rats (CNT). When plasma hGH levels were measured by repeated blood sampling every 20 min from 1000 h to 1800 h in 4 month-old male PGGB dr, hGH secretion was pulsatile with peaks, occurring every 3–4 hours as did rGH in male CNT. In contrast, in female PGGB dr hGH secretory peaks occurred at irregular intervals with higher basal hGH levels, as did rGH in female CNT. The present study indicates that the hGH gene is expressed specifically in the pituitary resume pulsatile GH secretion and normal growth in SDR.

Key words: spontaneous dwarf rat (SDR), targeted expression of the human GH transgene to the pituitary, normal recovery of growth and pulsatile human GH secretion

Introduction

A strain of spontaneous dwarf rat (SDR) with isolated complete GH deficiency of autosomal recessive inheritance (gene symbol dr) was discovered in Japan (1), and has been well characterized by others (2, 3) and ourselves (4–6). SDR produces characteristics similar to those of the human disorder, isolated GH deficiency Type I. We treated SDR by germline incorporation of a chimeric gene of the mouse metallothionein-1 gene promoter and human GH gene, which was ubiquitously expressed in SDR and resulted in rat gigantism (7).

Our previous study in hGH-transgenic rats has shown that a chimeric gene (designated as PGGB) of the rGH gene 5'-flanking promoter (gene segment 1.7 kbp upstream from the start point of
the rGH gene transcription) (8) and the genomic hGH gene (2.1 kbp) was required to increase the hGH transgene expression of the pituitary to levels approaching that of the rat GH gene (9). We have recently demonstrated successful treatment of the dwarf phenotype in SDR by targeting expression of the human GH gene to the pituitary gland of SDR (designated as PGGB-dr) (10). In the present experiment, we characterized the secretory profile of human GH secretion in both male and female PGGB-dr.

Materials and Methods

A chimeric gene (PGGB) of the rat GH gene promoter with human GH was first transmitted from PGGB-bearing Sprague-Dawley rats (PGGB-SD) (9) to SDR by crossing a male PGGB-SD (Tg ID#2846) with a homozygous female SDR (dr +/-, dr ID#322). A line of both male and female PGGB-bearing transgenic SDR (PGGB-dr) was established by backcrossing, and maintained by measuring both rGH and hGH levels in plasma under light ether anesthesia. The second and third generations of PGGB-dr were used for the analysis of body growth. Aged and sex matched SD (CNT) and SDR were used as controls. Individual rats in each group were weighed at weaning on day 21 and thereafter at 10 day intervals until 2 months of age.

Both CNT and PGGB-dr rats of the third and fourth generations were used for repeated blood sampling. Indwelling silastic cannulae were chronically implanted into the right atria of both CNT and PGGB-dr as previously described (11). Individual CNT and PGGB-dr rats were handled and weighed daily until body weight and behavior had returned to preoperative levels. The day before the experiment, the rats were placed in individual cages designed for injection and blood sampling while undisturbed and freely moving. Heparinized blood samples (150 μl) were obtained every 20 min from 1000 h to 1800 h, immediately centrifuged, and the plasma separated and stored at −30°C until assayed for both rGH and hGH.

![Graph](image)

**Fig. 1** Postnatal growth curves in SD, dr and PGGB-dr of both sexes. The means ± SEM are shown. The number of animals in each group is shown. Growth in PGGB-dr is indistinguishable from that in SD, but significantly greater than that in dr (dr v.s. PGGB-dr or SD, p<0.001).
Fig. 2 Representative spontaneous rGH, hGH and rPRL secretory profiles obtained from two individual males (a) and females (b) of CNT and PGGB-Tg. Blood samples were collected every 20 min from 1000 to 1800 h. Open circles and closed circles represent plasma rGH and hGH levels, respectively.
Group differences in growth curves of both sexes were respectively subjected to a one-way analysis of variance with repeated measures by means of a computer program StatView 4.5 J for the Macintosh computer. A probability of \( p < 0.05 \) was considered significant.

**Results and Discussion**

The postnatal growth curves in each group are shown in Fig. 1. Growth in PGGB-dr was indistinguishable from that in nontransgenic litter mates, but significantly greater than that in SDR. The dwarf phenotype of SDR was completely corrected by the targeted incorporation of the hGH transgene into the pituitary gland of SDR.

Both Northern blotting and RT-PCR analyses showed that the transgene transcript was expressed exclusively in the pituitary (data not shown). In spite of the lactogenic action of expressed hGH, the litter sizes of PGGB-dr were identical to those of age- and sex-matched nontransgenic littermates.

Sequential blood sampling from individual PGGB-dr under light ether anesthesia at day 20, 40 and 50 after birth showed age-related increases in plasma hGH (data are not shown), as did plasma rGH levels in each normal Sprague-Dawley rat (CNT).

As shown in Fig. 2a, rGH secretion in control male animals was pulsatile, with spontaneous GH secretory bursts occurring regularly at 1100–1300 h and 1400–1600 h in individual animals. In male PGGB-dr spontaneous hGH, but not rGH, secretory bursts occurred at the same time of day as in CNT. In contrast, as shown in Fig. 2b, rGH secretion in female CNT was episodic, occurring irregularly with higher basal levels than those in male CNT. In female PGGB-dr, episodic hGH secretion occurred irregularly. No endogenous rGH was detected in PGGB-dr throughout the experiments.

Thus we established a line of transgenic rats which are deficient in endogenous rGH, but grow with hGH indistinguishably from control SD rats. Our previous attempt to correct the dwarf phenotype of SDR by germline incorporation of a chimeric hGH gene coupled with the mouse metallothionein I promoter has resulted in rat gigantism (7) as reported in a strain of dwarf mouse (12). The mouse metallothionein I promoter-driven hGH gene was ubiquitously, but not in the pituitary, expressed in the giant SDR. There were no differences in hGH gene expression, as reflected in plasma hGH levels, between prepubertal and adult rats, or between males and females. By contrast, the hGH gene targeted (PBBG) to the pituitary resulted in normal recovery of both growth and pulsatile GH secretion in SDR of both sexes, suggesting that the strain of PGGB-dr has physiologically regulated hGH gene expression in response to the onset of puberty and sexually dimorphic growth rates (13).

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**References**


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